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Thesis

THE CHROMATOPHORE ACTIVATING SUBSTANCE IN THE PITUITARY GLAND OF THE VERTEBRATES.

by

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INTRODUCTION

Probably as long as man has had some sort of intelligence, he has noticed that the animals in his environment sometimes change in color. Undoubtedly, thinking men began to consider this problem, the problem of why and how these changes occur, long before there was any science. It is quite apparent that all these alterations of tint could not be of the same origin. The tanning of a man who has been exposed to the sun, the white winter coat of some mammals and the "wedding dresses" of certain fishes are certainly not the same. This paper will concern itself with color changes in the three lower classes of the vertebrates, namely, the fish, the amphibia and the reptiles. We shall endeavor to find not only what these changes are and how they occur, but especially what causes them.

A scientific treatment of this problem immediately presents two questions to be investigated. The first is: What part of the animal has a role in the color changes which we see? The second, which follows logically, is: Exactly what happens to this part of the animal during a color change? It is expedient in this paper to reverse the normal order of the questions and discuss the second one first. This is necessary because the latter question helps to define some of the terms which are needed to discuss the first one.

We have stated the second question in a general form above. Let us now restate it in the specific terms of this problem.

Fig. 1. A group of melanophores which have been expanded in a 0.1 molecular sodium chloride solution. (Obj. 8 mm Zeiss Apochrom., Oc. 6 Zeiss Comp

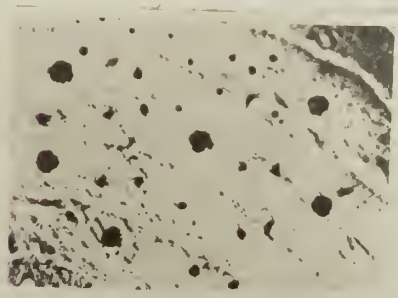


Fig. 2. The same melanophores contracted in a solution containing 4 cc. of 0.2 molecular potassium bromide + 2 cc. of 0.1 molecular sodium chloride.

g. 3. The same melanophores reexpanded in a 0.1 molecular sodium solution. The processes are identical with those appearing in the first figure. making these photographs no cover-glass was used. The illumination was

(Spaeth, 1913)

FIGURE 1

We must first be told that the chromatophores are activated and that this causes the color changes. Our revised question now becomes the heading of the next portion of this paper.

WHAT IS ACTIVATION OF THE CHROMATOPHORES?

Because there is no longer any serious controversy on this subject, it will not be discussed at too great length. Von Wittich (1854) observed the changes in the color of the skin of the frog and proposed that the differences in tint were due to an expansion and a contraction of the melanophores. He said the pigment cells were like amoebae and changed shape bodily. In other words, these cells although fairly fixed in location, could send processes out between other cells. There was little objection to this concept until Spaeth (1913) photographed an expanded melanophore in the scale of Fundulus, made it contract by chemical means and rephotographed it. Figure 1 shows the same scale in both conditions. This was repeated again and again. By comparing the processes of the same cell in the expanded state after several intervening contractions, he came to the conclusion that the processes are fixed and the apparent changes are due to the migration of melanin granules, since the outline of the cell was always identical.

Smith (1916a, 1916b) presented the theory that the melanophores of lighter tadpoles actually contained less pigment than those in dark animals. Other workers have substantiated this, but since it is apart from the main point of this discussion, it shall not be treated any further at this time.

Spaeth (1916a) gathered more evidence when he saw clear protoplasmic processes which extended far beyond the clumped melanin in a "contracted" melanophore, and in some of these processes, he saw the nucleus of the cell which was not in the in the central portion of the cell where the pigment was located. However, despite this evidence, Spaeth (1916b, 1916c) still spoke about contraction and expansion of the melanophores. As a result of this failure on Spaeth's part to give his discovery practical value by renaming this process, not only did others continue to use the old terms, but many of the were still not sure of the actual process and reinvestigated the problem.

Atwell (1919) in his observations on the tadpole said "chromatophores could be seen to expand slowly by sending out pseudopodia-like processes into which pigment could be seen to stream"

Hewer (1923) studied the method of melanin dispersal in the frog. He found three things which he thought were strong evidence toward the proof that the position of the pigment of these cells does not coincide with the cell boundary. They were: "The presence of 'frayed' ends to the processes, isolated granules and irregular edges to the concentrated mass of granules," He thought that these precluded any theories of amoeboid movement of the melanophores. The processes to which he refers are really the melanin granules entering the protoplasmic processes. He also felt that the irregular movements of the granules and the slight massing of the granules toward the tips of the processes in the dispersed phase supported the above evidence. This seems likely since these conditions would not be

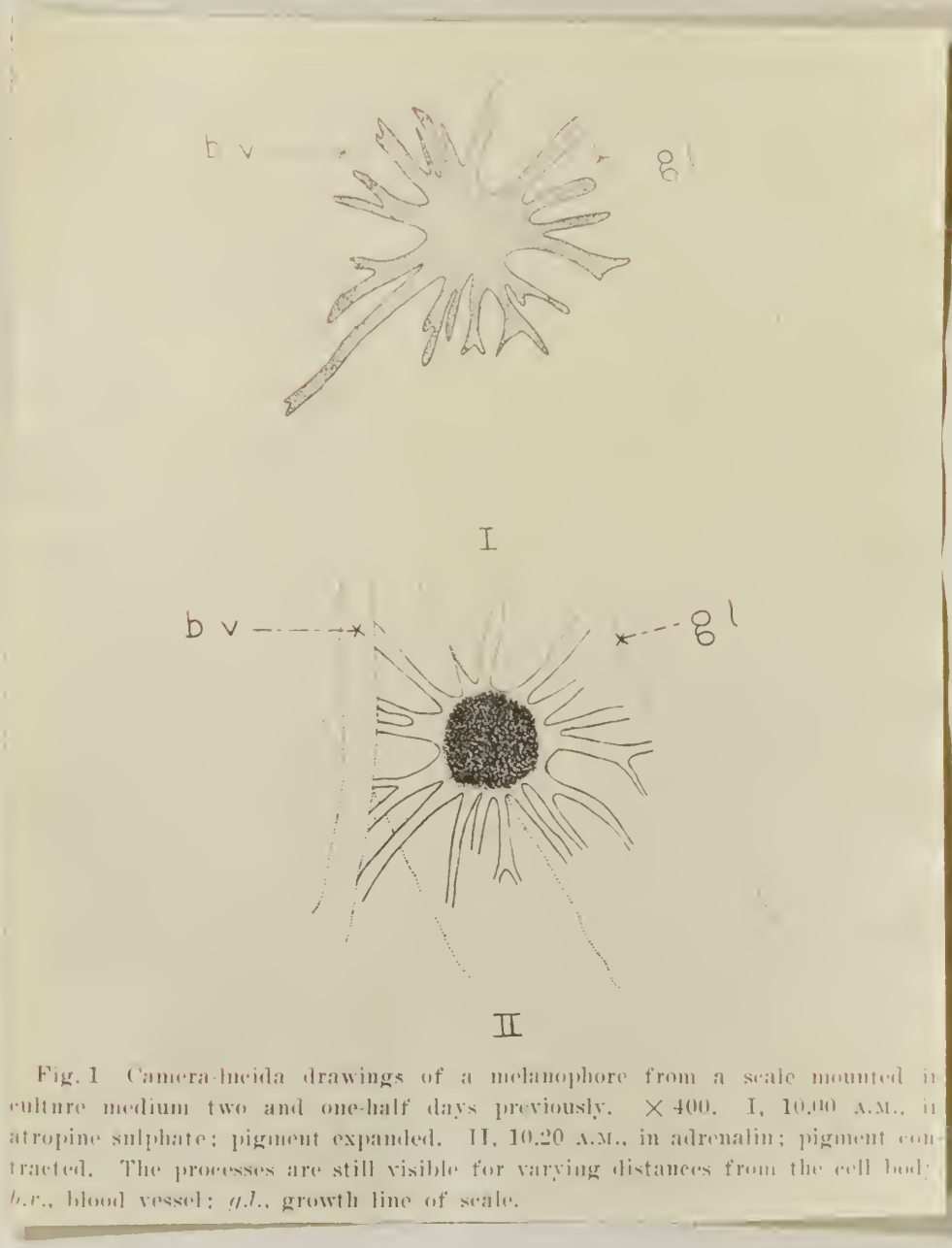


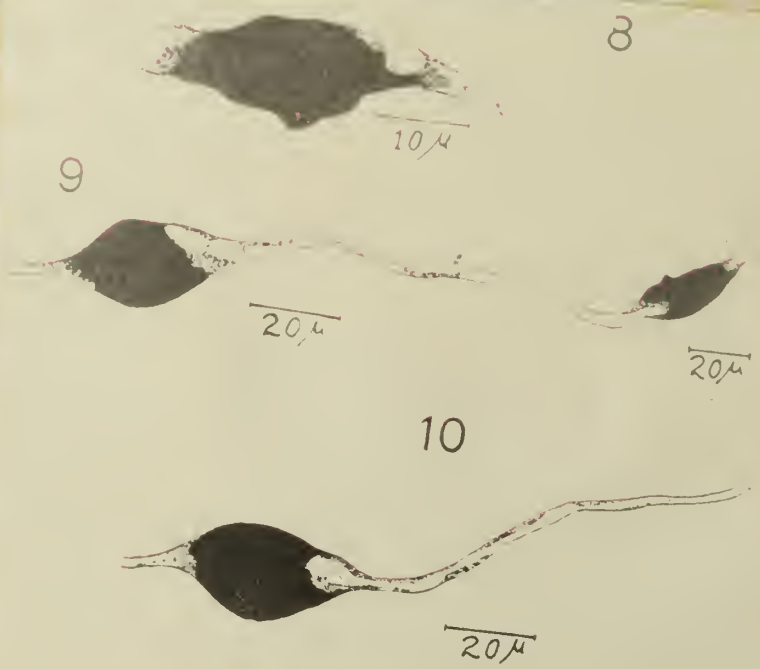
Fig. 1 Camera-lucida drawings of a melanophore from a scale mounted in culture medium two and one-half days previously. × 400. I, 10.00 A.M., in atropine sulphate; pigment expanded. II, 10.20 A.M., in adrenalin; pigment contracted. The processes are still visible for varying distances from the cell body; *b.v.*, blood vessel; *g.l.*, growth line of scale.

(Matthews, 1931)

FIGURE 2

present in amoeboid movement. Hewer's work was inconclusive because he could not see clear processes in the living animal. As a matter of fact, although Spaeth (1916a) saw part of the clear processes, this had been the main difficulty in all the investigation along these lines. The processes could be demonstrated in fixed material with appropriate staining, but not in vivo.

Finally, Matthews (1931) devised a method by which they could be seen. He made tissue cultures of Fundulus scales and, strangely enough, the processes then became readily visible. Some of his results can be seen in figure 2. "These processes are composed of homogeneous or possibly a very finely granular protoplasm with a delicate limiting membrane. An occasional nucleus may be seen in the processes in either the central or peripheral migration of the pigment granules." This work confirmed the observations of Spaeth (1916a). It showed further that there was no change in the diameter of the processes whether the pigment was located centrally or peripherally. Another interesting observation, although it is not entirely relevant here, is that the pigment in a melanophore process isolated from its cell by means of a microneedle can still spread itself over a wide field or concentrate in a small area even when the body of the cell has been completely destroyed. He also teased the melanophore with a microneedle both in the "expanded" and "contracted" conditions and found that in the former condition, the cell is more fluid and less brittle than in the latter. From the above results, Matthews drew the fol-



EXPLANATION OF PLATE III

FIG. 7. Camera lucida drawing of cell body of contracted epidermal melanophore showing nearly all of the pigment contained within the cell body. From living, uninjured tissue.

FIG. 8. Camera lucida drawing of contracted epidermal melanophore of frog tadpole showing processes devoid of pigment. From living, uninjured tissue.

FIGS. 9 and 10. Camera lucida drawing of nearly contracted epidermal melanophore of frog tadpole showing scattered pigment granules in the processes. From living, uninjured tissue.

(Herrick, 1933)

FIGURE 3

lowing conclusions: "1. The changes which the melanophores of Fundulus undergo when their widely dispersed pigment granules are concentrated in small areas are due to an intracellular migration of the granules and cannot be attributed to an amoeboid activity of the cell. The melanophnores not only do not withdraw in an amoeboid fashion but they do not contract laterally. 2. The fact that the pigment granules in a process which has become or has been isolated from its cell are still capable of clumping in a small area or of spreading out over a wide field indicates that the centrosphere plays no part in this activity of the granules, at least in the isolated process, and probably has little to do with the migration of the pigment in the intact cell. 3. The change in physical condition of the melanophore during central migration of its pigment granules tends to support Spaeth's hypothesis that the melanophore consists essentially of a colloidal suspension of melanin granules in a fluid sort of protoplasm and that the contraction of its pigment is brought about by a process similar to the gelation of an emulsoid." It is well to note here that, although the tissue which is being cultured is, naturally, not identical with normal cells, the melanophores behaved in exactly the same manner as in normal scales.

Herrick (1933) worked with tissue cultures of frog tadpole skin and found essentially the same things as Matthews. He, too, could see the clear processes (figure 3). He said that pigment granules in going into the processes did not migrate at the same speed as adjoining granules, but seemed to move in-

dependently and the pigment in the ends of processes was rather scattered and in no way suggested a pseudopodium of an amoeboid cell. He, too, concluded that "expansion" and "contraction" are accomplished by the migration of pigment granules into and out of permanent processes. These two papers seem to have proven conclusively what all the previous careful work had suggested.

Probably on the basis of this, Sumner (1935) felt the need of new terminology. He stated that while most investigators recognized the real action of the chromatophore, they still insisted in talking about the contraction and expansion of the chromatophores. He therefore proposed the terms "contraction" and "expansion" of the "chromatosomes" or pigment masses in the cells. Almost immediately, Mast (1933) objected to this since he did not think there was any evidence to support the theory that there was any pigment body which expands and contracts. He suggested the use of the phrase, "distribution and aggregation of the pigment granules."

Although it is easily seen that it is less difficult to talk about expansion and contraction of the chromatophores than distribution and aggregation of the pigment granules, the latter is accurate while the former is not. For this reason, this paper will employ the latter terms, but dispersion, scattering or diffusion will be used synonomously with distribution and concentration will be interchangeable with aggregation. Even at this date, not every paper on this subject has been weaned from the old terms. It will be noticed that the investigations which

have been cited above deal only with the melanophores. It must no be implied from this that they are the only chromatophores in the cold blooded animals. There are also silvery white cells, leucophores; golden white cells, xanthophores or xantholeucophores; red cells, erythrophores; and yellow cells, guanophores or iridocytes. Lipophores is a term sometimes employed to designate both xanthophores and erythrophores. Although no work has been done on the process of pigment movement in these cells because they are much more difficult to observe than the melanophores, we shall use the same terms in describing changes in all of them. This is logical, but it must be remembered that there is no proof for this assumption that all the chromatophores make their color changes in the same way.

WHAT IS THE ROLE OF THE CHROMATOPHORES IN COLOR CHANGES?

Now that some of the necessary terms have been discussed, let us return to the first question and inquire: What part of the animal has a role in the color changes which we see? For one of the early works on this question, we can return to von Wittich (1854) who concluded from his observations that the color changes of the frog were caused by the melanophores in the integument. This was accepted by most workers until Smith (1916a) started a controversy which initiated a good deal of work along these lines. Smith had hypophysectomized tadpoles and he noticed that they became light gray in color instead of brown-black colored. He said that the counts of melanophores of the lighter tadpoles showed fewer of these cells than corre-

sponding areas in the controls. He called the lighter animals "albinos". He said that the melanophores contained fewer pigment granules in the albinos, but that the pigment was equally dispersed in both animals. He also reported a scarcity of free pigment granules in the superficial layer of the epidermis, but just as much or more subcutaneous pigment.

Allen (1916), also hypophysectomizing tadpoles, found that the operated animals assumed a uniform creamy silver color after seven or eight days. He thought that the lighter color was due to a migration of the epidermal pigment cells to deeper positions and that the pigment was aggregated in the melanophores through out the body of the albino. He disagreed with Smith (1916a) and was convinced that there was "no disappearance and bleaching of the pigment granules as asserted by Smith." Allen (1917) later confirmed his own work and stated that "the removal of the anlage of the anterior lobe of the hypophysis early causes the pigment cells to contract and those of the epidermis to withdraw from it to the interior." Although he mentions removal of the anterior lobe, this is not quite accurate since he removed all the parts which are derived from the integument.

Atwell (1919) doing the same sort of work said that the silvering following hypophysectomy of tadpoles was due mainly to the concentration of pigment in certain melanophores and not to any marked reduction of pigment material present.

Smith (1919a) said that there were three main factors which caused albinos. In the first place, not only were there fewer

melanophores, but those that were present had less pigment and this was aggregated in the center of the cell. Secondly, there was a reduction of the so-called free melanin granules in the epithelium and, finally, the xanthophores in the deep and superficial strata of the dorsum of the head and body were always "expanded." This was the first time that anyone had attached any significance to these latter pigment cells. Smith said that, in normal conditions, there was a reciprocal alteration between the malanophores and the xantholeucophores. In other words, if the pigment of one were aggregated, the pigment of the other would be dispersed. He refuted Atwell's (1919) statement that the darkening was due to the concentration of the pigment in the dermal melanophores since these were covered by the xanthophores. When the latter cells were in the dispersed phase, the pigment cells beneath them were invisible. In further refutation, Smith stated that while the great reduction of the so-called free pigment in the superficial layer of the epidermis has no more than a minor role, it is just as striking and constant as other pigment effects. One reason why Atwell may have overlooked this point is that many fixatives have a solvent action on these cells. Smith also added that " the widely expanded xantholeucophores of the albinos are singularly unanswerable to most experimental influences and in this they are strikingly in contrast to iridescent cells of the normal animals." Smith (1919b) later confirmed his work and said that while the pigment of the epidermal melanophores can be made to come to the aggregated phase and the xantholeucophores to the

dispersed phase by subjecting normal tadpoles to strong light and warmth, this does not occur to the degree found in hypophysectomized animals. He made one more statement which seems a little strange. He said that when all stimuli are removed, the resulting conditions of the pigment cells do not differ greatly in the normal and albinous larvae. This is peculiar since these animals can easily be differentiated from each other on casual observation. Smith (1920) further substantiated his previous claims. He definitely proved, in a series of experiments, that the condition of the dermal melanophores was insignificant in the coloring of the albino because the xantholeucophores made them invisible in this animal.

Atwell (1921) contested the work of Smith once again. He still held that while the epidermal pigment conditions were as Smith had said, the xantholeucophores gave the albino its metallic sheen. Furthermore, the dermal melanophores were an important factor in the light color of these animals. He proved this by giving the albinos pituitary feedings. This had not much effect on the restoration of epidermal pigment, but caused a marked expansion of the dermal melanophores which produced a definitely darker animal. The xantholeucophores were unaffected. Incidentally, Smith had tried to duplicate these experiments and failed to do so.

Swingle (1921) took the next step in this line of experimentation and implanted pituitary glands from adult frogs in hyposectomized tadpoles. His result showed a reversal of the conditions found in the albino. The pigment of the melano-

phores dispersed while that of the xantholeucophores concentrated in the center of the cell. In the albino, he agreed that the dermal melanophores had their pigment in the concentrated state, although the xantholeucophores probably make any change in these cells invisible.

Wyman (1924) was of the opinion that the normal color changes in Fundulus were due to a change in the pigment distribution in the melanophores in this fish.

Houssay and Ungar (1924a, 1924b) said that the color change in frogs and toads was due to an aggregation of the pigment of the melanophores and converse distribution of the pigment of the guanophores in the hypophysectomized animal. In the frog there was an apparent migration of the lipophores in addition. Giusti and Houssay (1924) agreed with this when they said that the color changes in a hypophysectomized toad was caused by an aggregation of the pigment in the dermal melanophores, a dispersion of pigment in the guanophores and migration of the lipophores. When these latter authors speak of guanophores, they probably refer to the same chromatophores as the others who use the term xantholeucophores.

Our problem here, is to decide, not the relative importance of different types of chromatophores in producing color changes, but whether or not they cause the color changes which we see. All of the workers above have been unanimous in their agreement of that fact and almost every other paper mentioned here, assumes or prooves this. There can be no doubt that in the cold blooded animals, color changes are effected mainly by alteration

of the chromatophores and perhaps to a very slight extent to the free pigment in the integument.

The next step leads us to the body of this thesis. We have found that the chromatophores of the integument cause the color changes in the cold blooded animals and that the alteration of these pigment cells is due to a redistribution of the pigment which they contain. Our next line of discussion will be: What factor or factors in the animal cause the chromatophores to react? For the sake of clarity, we shall consider each class separately.

PRESENCE OF A CHROMATOPHOROTROPIC SUBSTANCE IN VERTEBRATES

Pisces

Among the elasmobranchs, Houssay and Ungar (1924b) found that extracts of the pituitary gland of the ray, Raja platana, caused the pigment of the melanophores of the frog integument to disperse. Not only did the whole pituitary extracts give this effect, but, to a lesser degree, so did extracts of the infundibular lobe. Unfortunately, these men did not investigate the effect of the these extracts on the dogfish melanophores. Therefore, the only thing we can conclude from their work is that there is something in the pituitary gland of the ray which disperses the pigment of the melanophores of the frog.

Lundstrom and Bard (1932) went further and hypophysectomized the dogfish, Mustelis canis, and found that this caused pallor

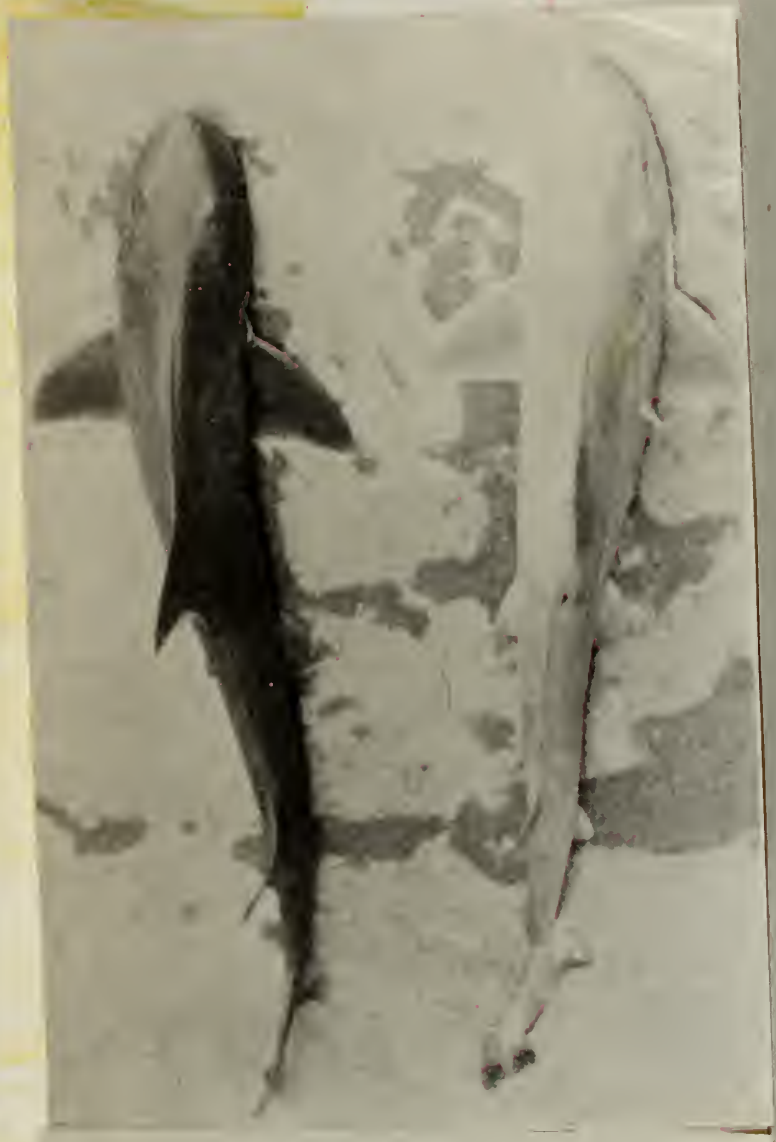


PLATE 1. Photograph of a pair of dogfish, originally of the same shade, taken twenty-four hours after removal of the hypophysis in the animal on the right. Both animals had been kept in the same tank and the operated individual showed no abnormalities of behavior.

(Lundstrom and Bard, 1932)

FIGURE 4

of the skin of the fish (figure 4). This was the result of a concentration of the pigment of the cutaneous melanophores which is usually in dispersed phase. By injecting pituitary extracts into these fish, either intramuscularly or subcutaneously, they were able to produce fish of normal color. They also covered portions of the skin of the normal fish and subjected it to strong illumination. The entire fish became lighter, even under the patches. These experiments show that the melanophores do not react directly to light and that a hormone in the hypophysis has an effect on the melanophores of the dogfish. These investigators also tried to get the pigment of the melanophores in a hypophysectomized fish to disperse by putting the fish in a dark container for some time. Since a normal fish will become darker and this fish did not, they concluded that the pituitary in the dogfish plays a dominant role in the color changes of the normal dogfish. Their work did not preclude the possibility of some other control of the cutaneous pigmentation.

Parker (1935a, 1935b) stated that the dark phase in the dogfish is caused by the pituitary secretion, but that the light phase is controlled by the nerves and can not be excited by the blood from a light fish.

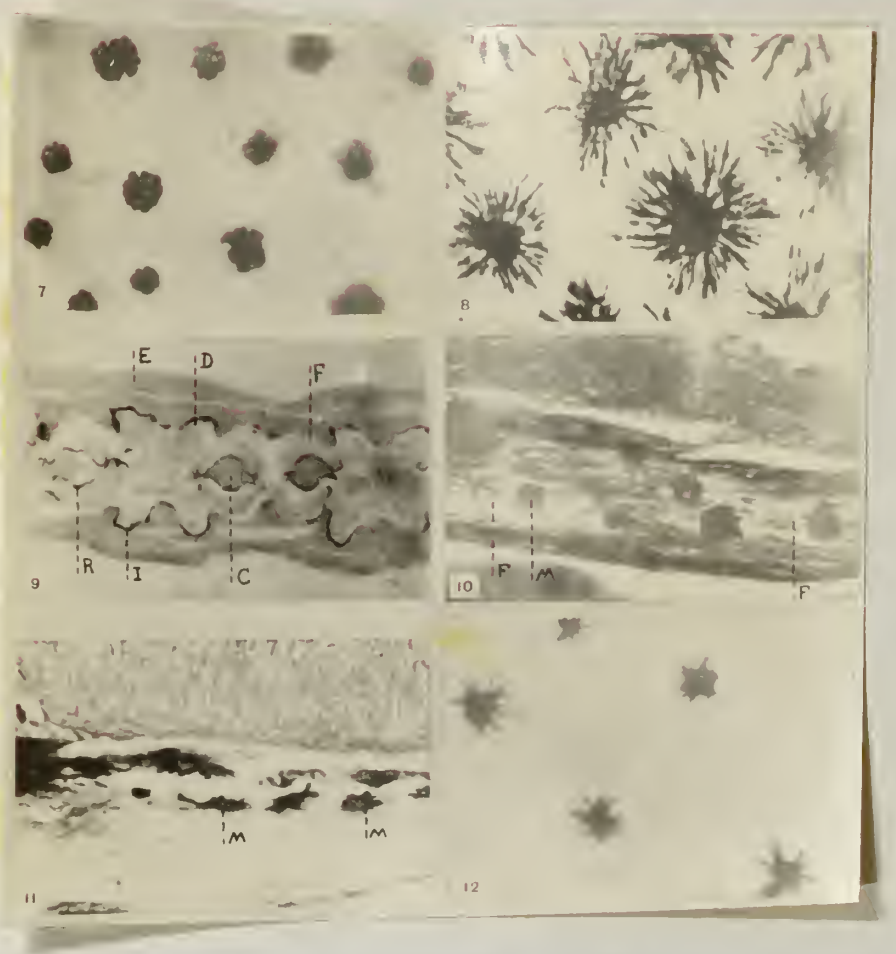
Veil and May (1937) worked on the torpedo, Torpedo marmorata, and concluded that the neurointermediate lobe of the pituitary gland plays an indisputable role in the cutaneous pigmentation of the torpedo. They injected several cubic centimeters of blood from a normal torpedo into a hypophysectomized

one, but it did not contain enough of the pituitary hormone to darken it.

This may be a partial refutation of Parker's (1935a & 1935b) work because he based part of his conclusion on the fact that blood from a light fish did not have any effect on a dark one. From that experiment he concluded that the light phase could not be governed by anything soluble in the blood. It may be only as Veil and May concluded that the substance was not present in sufficient quantity. However, the last named workers inferred from their experiments that in the normal state the distribution of the pigment in the melanophores of the torpedo is due to the irrigation of these cells by blood containing the minimum amount of the pituitary hormone, but constantly renewed.

Barry (1937) found a light dogfish in a group of normal fish. He injected it with ox pituitary extract from the posterior lobe. When given in sufficient quantity, this caused the fish to darken. The pituitary of this fish was slightly abnormal, but in histological section it appeared normal.

Vilter (1937) attempted to show that the control of the melanophores in the elasmobranchs was under a double physiological control. He felt that the pallor which follows hypophysectomy was due to a stimulation from the sympathetic fibers. In the rays, Trigon pastinaca and Raia undulata, which had their pituitaries removed, he was unable to disperse the pigment of the melanophores by the injection of 0.1 to 0.2 cc of ergotamine. This he felt was caused by the paralysis of the terminal fibers of the drug.



7. Microphotograph of completely contracted melanophores of Fundulus.

8. Microphotograph of completely expanded melanophores of Fundulus.

9. Microphotograph of a tranverse section of the caudal fin of Fundulus. E, epidermis; D, dermis; F, dermal fin rays; C, connective tissue and bloodvessels in fin ray; I, interradial melanophores; R, radial melanophores.

10. Microphotograph of a sagittal section of the normal tail of Fundulus with the nerve fibers stained. M, melanophores; F, nerve fibers.

11. Microphotograph of a sagittal section of the denervated area in the tail shown in figure 10, treated with the same technique. M, melanophores.

12. Microphotograph of denervated melanophores of Fundulus, showing the stellate condition.

(Wyman, 1924)

FIGURE 5

These investigations have shown that the pituitary plays a large role in the color changes of the elasmobranchs. Whether or not this is the sole controlling factor has not been proven.

In 1918, Spaeth wrote a paper describing a method for the biological standardization of pituitary extract. He used the isolated scales of the minnow, Fundulus heteroclitus, to test a commercial pituitary extract, namely, Pituitrin (Parke Davis). The scales showed a concentration of the pigment of the melanophores. Wyman (1924) investigated the melanophores in the intact Fundulus and found that the pigment reacted in the same way to injections of desiccated pituitary gland; however, he stated that these glands were not reliable for use in physiological experimentation (figure 5).

Abolin (1925) found that injections of Infundin, a commercial pituitary extract produced darkening in Phoxinus laevis. In other words, the pigment of the melanophores had been dispersed. Hewer (1926) produced results which were exactly the opposite of those of Abolin. Although it was thought that the reaction of the isolated scales differed from that of injections into the intact animal, it should be noted that these results, namely, a concentration of the pigment in the melanophores, agree with the results of both Spaeth (1918) and Wyman (1924). The results of the investigations differ so greatly that Hewer (1926) suggested that the pituitary extracts used may not all be identical. He also found that in the normal state, the pigment in the erythrophores, xanthophores and the melanophores reacts in the same way, but pituitary extracts cause the pigment of the

Abb. 1. *Unten*: Bitterlingsmännchen vor der Einspritzung. Das Tier ist hell graugrünlich gefärbt. An den Flossen ist keine Spur von Rötung zu erkennen. *Oben*: Bitterlings-männchen mit deutlicher Hochzeitsfärbung, wie sie bei Einspritzung von 0,1 ccm Extractum testiculi HENNING in etwa einer halben Stunde zu sehen ist. Der Rücken ist blauschwarz gefärbt. Die Rücken- und Afterflosse hat einen schwarzen Rand. Die Rötung in einem kleinen Winkel an der Rückenflosse und vor allem an dem größten Teil der Afterflosse tritt deutlich in Erscheinung. Auch auf der Brust- und Bauchgegend treten rote Flecken auf und die Iris ist gerötet. Die Färbung gleicht vollkommen der natürlichen Hochzeitsfarbe. Während diese jedoch nur für Minuten im Augenblick höchster geschlechtlicher Erregung in Erscheinung tritt, hält die experimentell erzeugte Hochzeitsfärbung stunden- ja tagelang an.

(Wunder, 1931)

FIGURE 6

melanophores to concentrate and that of the erythrophores and xanthophores to disperse. From this, Hewer concluded that the posterior pituitary extract does not take part in the normal reactions of the melanophores, erythrophores or xanthophores.

Smith (1931) could find no hormone which would cause dipersion of the pigment of the melanophores. This work was done with Phoxinus. Giersberg (1931) found that injections of Infundin caused the pigment of the melanophores to disperse in this same fish. This obviously disagrees with Hewer's findings.

Wunder (1931) worked with the bitterling, Rhodeus amarus, and the stickling. In uncastrated males, he was able to produce the body coloring which corresponds to the "wedding-dress" of these two fish (figure 6). Naturally, these experiments could not be attempted during the spawning period. He found that feeding the hormone had no effect, hormones in the aquarium water a very slight effect and that injections were most effective. He did not attempt to say whether or not these conditions were similar to the normal ones.

Giersberg (1932) found that injuries of the Phoxinus pituitary cause a decrease in the color changes of the lipophores and extirpation causes elimination of color changes. Implantation of pituitary or injection of Infundin produces an uncontrollable yellow and red coloring by flooding the blood with pituitary hormone. He concluded that "the lipophores of Phoxinus are thus hormonally regulated and indeed the pituitary is the specific endocrine gland which conditions their expansion

by elimination into the blood of a color change hormone. There seems to be no sort of additional nervous control of the lipophores."

This same discrepancy of results is found in most of the work done in this field. For this reason, the following workers and their results will be merely briefly mentioned.

Osterhage (1932) found that injections of Hypophysin in Gasterosteus aculeatus and Rhodeus amarus caused dispersion of the pigment in the melanophores. Blanchard, Prudhomme and Simonnet (1932) got the same effect with injections of posterior pituitary extracts in Acerina cernua and Gobio fluviatilis. In males of 65 to 75 mm of the species Phoxinus laevis, Zondek and Krohn (1932a) produced dispersion of the pigment of the melanophores, xanthophores and the erythrophores by injection of intermedin. They found the same thing in Rhodeus amarus.

Odiorne (1933a) reviewed the literature and added some results of his own. He found that Pituitrin had no effect on either the isolated scales of Fundulus or upon injection. Pitressin caused the dispersal of the pigment of the melanophores in the catfish, eels and goldfish. Pituitrin has the same effect in these fishes. However, Antuitrin injections cause a concentration of the pigment of Fundulus. The same thing occurs with isolated scales. It seems probable from this work that the extracts which have been used were not of uniform composition in all experiments. Odiorne concluded that the melanophores of the majority of fishes have their pigment dispersed by Pituitrin. He also thought that "in Fundulus, the results previously at-

tributed to Pituitrin seem to be due to some principle contained in Antuitrin."

Collin and Drouet (1933), with posterior pituitary injections, were able to get concentration of the pigment of the melanophores and dispersion of the pigment in erythrophores and lipophores. Matthews (1933) found that in the isolated scales of Fundulus, hypophysial extracts cause distribution of the pigment of the xanthophores and aggregation of the melanophores. If the fish were hypophysectomized, it still responded as the normal fish to changes in the illumination and background.

Peczenik (1933) found that he could produce the "wedding-dress" in Phoxinus by injections of Pitophorin. Stutinsky (1934) found that Phoxinus females did not respond to doses of pituitary and in addition the reaction of males was not as constant as that claimed by Zondek and Krohn (1932a, 1932b). Collin and Drouet (1934b) replied to this that Zondek and Krohn (1932a, 1932b) had specifically stated the size and sex of the fish to be used because of variations in different fish.

The only conclusion that we can draw from the above investigations is that although the pituitary gland has certainly some effect on the chromatophores of the fish, it is probably not the only governing factor. In addition, the wide variation of results seems to point to the fact that the extracts which these workers used must vary greatly in their composition and purity. Most of the work in the teleosts has been in the minnow family. This is probably because of the wide distribution and hardiness of this fish. It certainly can not be said that the pituitary

Fig. 7 Photograph of an albino. × 2. Note the very small hind limb [illegible]
Fig. 8 Photograph of a normal tadpole. Figures 7 and 8 were photographed on the same plate. × 2.
Fig. 9 Photograph of a normal tadpole. × 2.
Fig. 10 Photograph of an albino. × 2.

(Smith, 1916a)

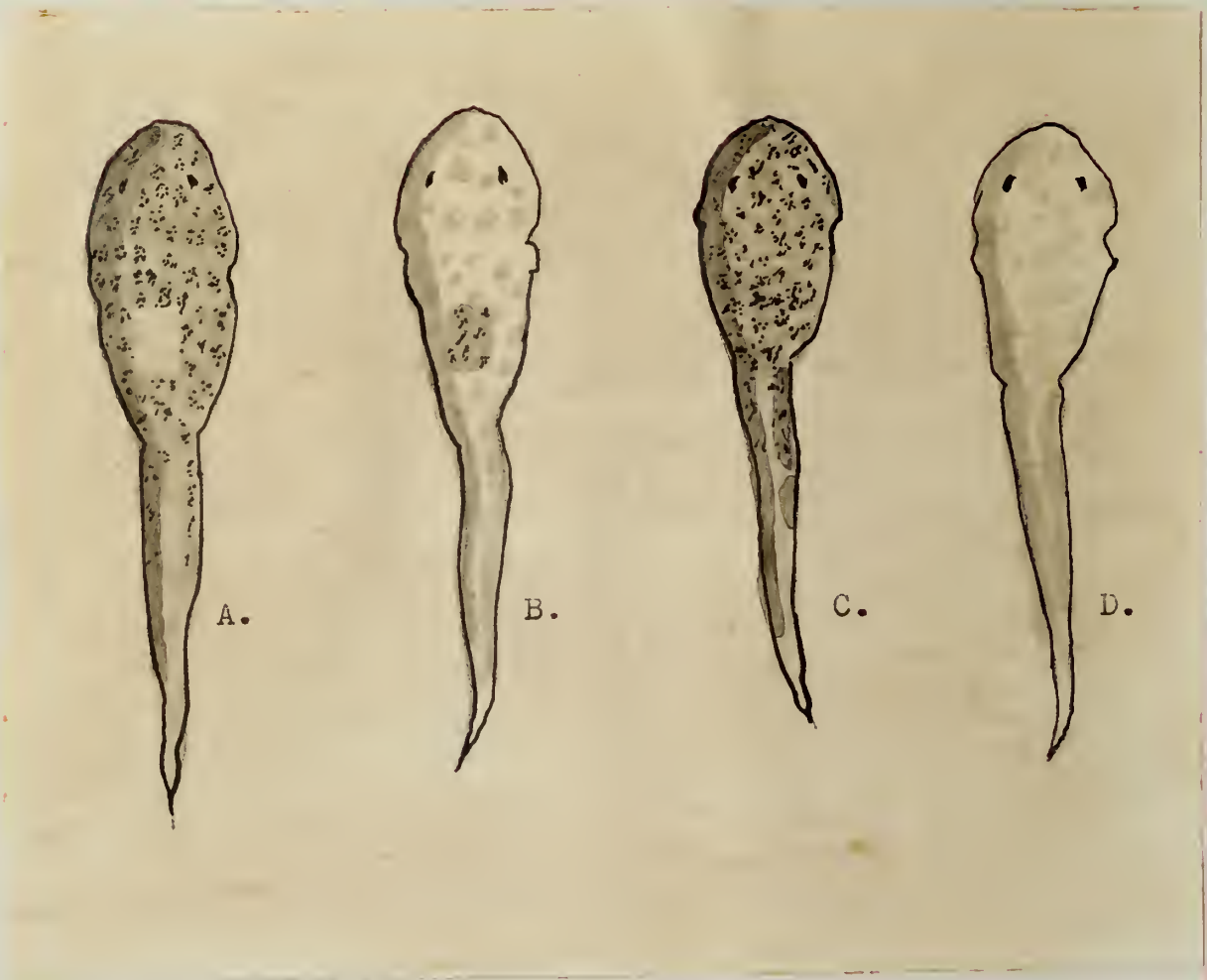
FIGURE 7

has a definite chromatophore hormone in all fishes, but in those which have been studied, such a hormone seems very likely. However, all the results seem masked by some other important controlling mechanism in the fish. Most investigators now feel that this factor is probably a nervous control through the sympathetic system. There may also be some antagonistic hormonal influence, as for example, adrenalin.

Amphibia

Although some of the following papers have been mentioned in the introduction, they will be cited here to present a more complete summary of the work in this class.

Smith (1916a) found the frog tadpoles grew lighter after removal of the pituitary gland. As we have mentioned, he thought this was due mainly to a decrease in the number of melanophores in the albino tadpoles. In this work and later, Smith (1916b) was of the opinion that the pigment in the cells was fully dispersed in both the normal and the experimental animal. Some of Smith's results can be seen in figure 7. Allen (1916), also also working on Rana tadpoles, stated that the pigment cells were in the "contracted" state in the albino. Allen (1917) again got the same results as in his previous work and further stated that some of the melanophores of the epidermis had migrated to the interior in the lighter larvae. Atwell (1919) worked on tad - poles of Rana sylvatica and observed that both the epidermal and the dermal melanophores were found to have their pigment concen-



DRAWING AFTER RESULTS OF SMITH, 1919c.

Tadpoles A and C are normal tadpoles having implants of integument from albino tadpoles. Tadpole A shows condition immediately after transplantation and tadpole C, three weeks after after transplantation.

Tadpoles B and D are albinous tadpoles having implants of integument from normal tadpoles. Tadpole B shows condition immediately after transplantation and tadpole D, three weeks after transplantation.

FIGURE 8

trated. By putting the silvery tadpoles into solutions of pituitary extract he was able to make the pigment of the melanophores disperse.

Smith (1919a) conceded that the melanophores, when they were present in the hypophysectomized tadpoles, had concentrated pigment. However, he stated that this was only a secondary cause for lighter color, since there was a great reduction in the number of pigment cells in these animals, as he had noted previously. Smith made the important discovery that the melanophores were not the only important pigment cells which were affected by removal of the pituitary. In the dermal layer of the integument there was a group of golden white cells, xantholeucophores, which were activated by hypophysectomy. The pigment of these cells was distributed throughout the cell in the albino tadpole. This not only gave the animal a metallic lustre but masked the corial melanophores. He was unable to make the melanin of the black pigment cells disperse by pituitary extracts as Atwell (1919) had done. Smith (1919b) confirmed his work and stated that the reaction of the xantholeucophores went hand in hand with the reaction of the melanophores, but had opposite direction.

In an attempt to find whether or not the migration of pigment in the xantholeucophores was under nervous control, Smith (1919c) made skin transplants from the dorsal area between albinos and normal tadpoles (figure 8). Successful exchanges altered the state of the pigment in these cells to correspond to that of the new host, i. e., the pigment was dispersed in the albinos and concentrated in the xantholeucophores of the unoper-

ated tadpole. Smith (1920) tried various pharmacological and physiological agents on the pigmentary system of normal and hypophysectomized tadpoles. He found that the dermal melanophores of both the normal and the operated animals behaved identically under all the tests he tried. While the xantholeucophores of the normal larvae respond to changes in the environmental conditions, those of the albino reacted only to pars intermedia emulsion. The pigment dispersed in this case.

Allen (1920) found that he could get albinous frogs to darken by implanting grafts of either intermediate or posterior lobe of the pituitary in these tadpoles. These implanted larvae became darker than the normal ones in the same enviroment. The coloration took ten days before it was complete and then persisted for a long time although not indefinitely. Atwell (1921), in further experiments on hypophysectomized tadpoles, found that the pigment of the xantholeucophores was dispersed in the albinos. This condition could be reversed although not completely by injections of posterior lobe extracts which also caused the pigment of the melanophores to disperse.

Swingle (1921) also concurred with the previous workers. He implanted pars intermedia in the tadpoles of the species Rana catesbiana, pipiens and clamitans. The grafts came from adult frogs and both heteroplastic and homoplastic implants were made. The hypophysectomized tadpoles regained their dark color and kept it, up to forty days after the implants were made. He found the pigment dispersed in the xanthophores of hypophysectomized tadpoles and in the melanophores of both the normal and

implanted tadpoles and concentrated in the xantholeucophores of the normal and engrafted tadpoles and in the melanophores of the of the hypophysectomized larvae.

Hogben and Winton (1922a) worked with adult intact frogs which had been adapted to a light background and kept dry. This insured maximum concentration of the pigment of the melanophores. Into these frogs was injected extracts of the pituitary of other frogs. This caused them to become darker, even in the same environment. Microscopic observations revealed the pigment of the melanophores of these frogs to be dispersed, while the pigment of the xantholeucophores in the dermal layer became concentrated. By dilution experiments, they further found that the extract of the pituitary of one frog was sufficient to darken the skin of fifty-six frogs. These investigators were able to obtain the same results with pituitary extracts on isolated frog skin. They thought that the skin might react with more constant sensitivity than the intact animal. They also found (1922b) that pituitary injections of extracts from the rat and the ox produced the same results as frog pituitary. To paralyze different types of nerve endings, cocaine (afferent), curare (spinal efferent), atropine (parasympathetic) and apocodeine (sympathetic) were injected, but subsequent pituitary injections gave the usual results. Curare, atropine and cocaine have no effect on the melanophores of the frog. Apocodeine darkens the skin, but not enough to make the pituitary effect unnoticeable. For this reason, Hogben and Winton concluded that "action on nerve endings in melanophores is ruled out."

Smith and Smith(1922) found that injections of fresh anterior lobe substance from an ox injected into hypophysectomized tadpoles gave an animal as dark as normal, but slightly more reddish.

Darkening of hypophysectomized tadpoles by various pituitary injections was found by Smith and Smith (1923a, 1923b). Hogben and Winton (1923a, 1923b) produced darkening in adult light adapted frogs. Hogben and Crew (1923) found that hypophysectomized axolotls became paler than the normal animal. They used the operated animal as a test for the pigmentary hormone in the ox and the sheep fetus and got positive results. Houssay and Ungar (1924a, 1924b) produced pallor by ablation of the pituitary gland in the frog, Hyla raddiana, and the toad, Leptodactylus occellatus. Pituitary injections in these animals caused distribution of the pigment of the melanophores even though the extracts came from many different animals in addition to the amphibians. Fenn (1925) devised a method for assaying the power of the pituitary gland to affect the chromatophores, which was based on a method of perfusion. The results found were similar to those above and the test animal was the frog.

Allen (1925), working on Rana pipiens and Rana aurora draytoni adults and larvae, and Van Dyke (1926), on frog adults, agreed with the previous workers. Collins and Adolf (1926) experimented with the vermillion spotted newt, Diemyctylus (Triturus) viridescens Rafmesque, but here the animals became lighter as the result of feeding or injecting pituitrin. Snyder (1928) obtained darkening of albino tadpoles and adults by pit-

uitary extracts. Marx (1929) hypophysectomized salamanders and found that although they get lighter immediately, later they darken spontaneously if there is even a small portion of the pars buccalis remaining. Allen got darkening of albinous tadpoles by pars intermedia transplantation in Rana pipiens (1929a and 1929b). He repeated this (1930) and was even able to get grafts of adult hypophysis to live in tadpoles with the dark color retained as long as the graft stayed alive. Hogben and Gordon (1930) returned hypophysectomized adults of the south African clawed toad, Xenopus laevis, to normal coloration by pituitary injections. Burns (1930) implanted the entire hypophysis of the adult Amblystoma trigrinum into young larvae of Amblystoma. The young axolotls metamorphosed and became darker as a result of the permanent establishment of the grafts. Klatt (1931) got similar results by pituitary implantation in hypophysectomized Triton larvae.

Witschi (1931) produced pallor by ablation of the pituitary in the Californian newt, Triturus torosus. This latter investigator worked with parabiotic pairs. He found that if the pituitary were removed from one of the pair, it became and remained lighter than its mate. He proposed that this was because the melanophore hormone was used up in the capillary network in passage from one animal to the other. It may be, however, that, as we have noted before, the pituitary does not produce enough of this hormone for more than one animal under normal conditions of secreting this substance.

Nobel and Richards (1932) implanted different parts of the

pituitary of the adult Bufo fowlerii into larvae and adults of the salamander, Pseudotriton ruber ruber (Soninni), with success. They found that anterior pituitary implants gave a distinct reddening of the animal while posterior and intermediate grafts gave darkening due to dispersion of the pigment in the melanophores. Collins and Drouet (1932) got the usual results with frogs injected with pituitary extracts from various animals. Koller and Rodewald (1933) found that pituitary extracts from the glands of dark adapted frogs did not cause hyphphysectomized or light adapted frogs to darken.

Dubowik (1933) got the usual pallor following hypophysectomy, but he claimed that the axolotls "repigmented" themselves after some time. Dietel (1933 a and 1933b) used the frog as a test animal to determine various methods of chemical extraction of the melanophore activating hormone. Drouet and Forentin (1933) used the frog to determine the amounts of this hormone in humans in various physiological and pathological conditions. Collins and Drouet (1933a) found that the frog reacted as usual to post pituitary extracts. They (1933b) also used it as a test animal for the substance. In 1934a they found that transplants of the pars anterior of the sheep caused an intense blackening in Rana temporaria adults which appeared in ten minutes and lasted for twenty four hours. Dietel (1934) used the frog to standardize a melanophore hormone extract. Jores (1934) found that he could get light adapted frogs to respond to the pituitary of dark adapted frogs if the extract were first treated with an alkali. Geiling and Lewis (1935) found that tissue cultures

of certain of the pituitary lobes produced enough hormone to darken light adapted frogs.

Teague, Noojin and Geiling (1939) tested many drugs in reference to their effect on the melanophores of the intact and hypophysectomized frog and found that only posterior pituitary injections were effective in causing distribution of the pigment of the melanophores in the hypophysectomized animals. Shen (1939) injected various drugs which stimulated the pituitary gland. This produced a marked darkening in the intact frog, but had no effect upon the pituitary-less frog.

The writer, in one experiment, after injecting light adapted frogs with whole pituitary gland from dark adapted frogs, produced a further pallor in the former group. In several succeeding experiments, in which the dark adapted frogs were kept in absolute darkness for over a week except for short exposures to red light, extracts from the pituitaries of these frogs still caused darkening in light adapted frogs.

Although we have discussed the effect of some pituitary substance on the chromatophores of the integument in amphibia, with special emphasis on the melanophores, these are not the only pigment cells affected by this substance. Swingle (1921) reported that the deep lying melanophores of Rana react as well. The cells containing melanin on the lungs, liver, peritoneum, pericardium and brain membranes react also, although not with such clear cut and striking results. Dubois-Poulsen (1925) found that pituitary extract causes the pigment of the retina to migrate to the external limit where it seems to be paralyzed,

that is, it does not react further to light stimuli. Jores and Caesar (1935) held that although application of the hormone in the light adapted frog had no marked effect, it caused the eye to adapt itself to the dark much more rapidly. The ablation of the pituitary seemd to have no effect. Matuo (1935) found that subcutaneous injections of posterior lobe extracts in the dark adapted frog produces a light arrangement of the retinal pigment cells, i. e., the pigment granules migrate to the limiting layer. However, the retinal cells still behave as in normal frogs after extirpation in dark and light adapted frogs. Therefore, Matuo concludes, that the pigment migration in light stimuli does not depend on the pituitary, but the hormone of the posterior lobe still has an effect.

From the experiments with amphibia, it can be seen that the pituitary has a major role in the dispersion of pigment in melanophores and concentration in the xantholeucophores. It is probable that there is little or no nervous control in the amphibia as there is in the fish. It is not yet certain whether the opposite effect in the chromatophores is merely a relaxation or an antagonistic effect of another hormone. Since the xantholeucophores and the melanophores react in opposite directions, it is improbable that the effect could be one of relaxation. However, there can be no doubt that in this class, there is a potent chromatophore activator secreted in the pituitary.

(Kleinholz, 1935)

1. On the right is a normal Anolis in the dark condidtion. The animal on the left has been hypophysecomized and is consequently in the green or light state.

2. On the right is a normal catfish that has been darkened by adaptation to a black background. The fish in the center had been light-adapted to a white background and then been injected with 12 Fundulus pituitaries; one hour after the injection it was photographed in this dark condition. The animal on the left is a control light-adapted fish that has received 0.5 cc of Ringer's solution intraperitoneally.

3. The frog on the right has been light-adapted and then injected with eight Fundulus pituitaries, rephotographed 75 minutes after injection. The animal on the left was the control that received an injection of Ringer's solution.

FIGURE 9

Reptilia

Surprisingly enough, even though the chameleon, which belongs to this group, is the most famous animal for its color changes, there has been very little work done in this class.

Houssay and Ungar (1924b) in their extensive investigations on the presence of the chromatophore activating substance in the vertebrates discovered that extracts of the tortoise pituitary produce a dispersion of the pigment of the frog. They did not study the effect of this hormone on the tortoise itself. Noble and Bradley (1933) attempted to hypophysectomize the lizard, Hemidactylus brookii, but it was not always complete. Of two with no hypophysis left, one remained light until death. Those with some pars anterior or pars posterior tissue left, but no pars intermedia, reamined light colored. Two with some pieces of pars intermedia remaining were darker than these last. One with some pars intermedia left, was still darker, and one, with the entire pars intermedia and the pars posterior present, was always dark, i.e. normal.

Kleinholz (1935) in testing the pituitary of Fundulus for a melanophorotropic substance found that an extract of this gland cause the melanin of the melanophores of the lizard, Anolis caroliniensis,to disperse. The same author (1938a) reported pallor following hypophysectomy in this lizard and produced the normal brown color by the use of pituitary injections (figure 9).

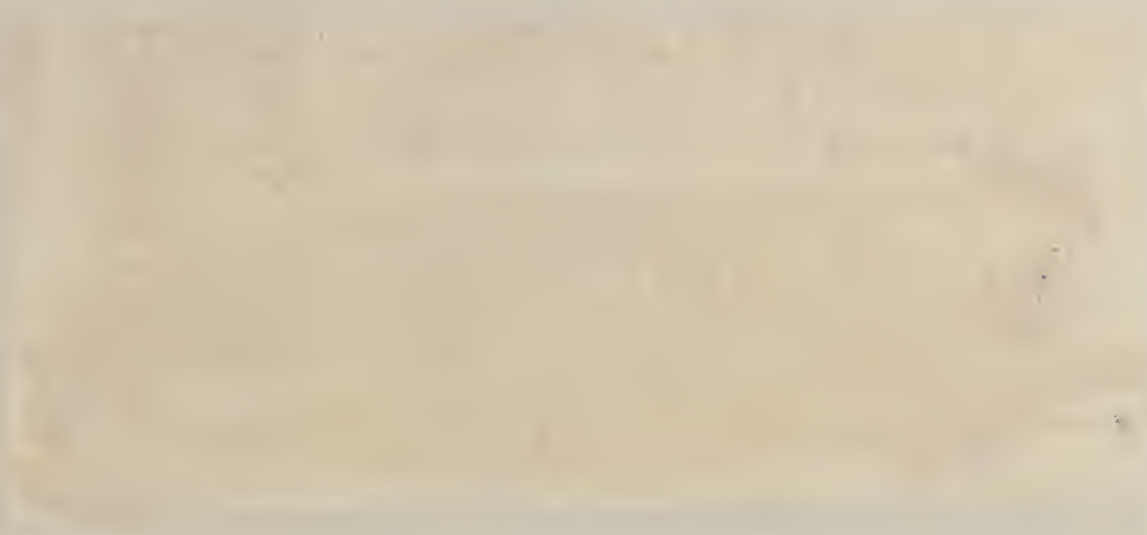
Kleinholz (1938b) said that the skin of the hypophysectomized

Anolis does not respond directly to light. He said that the concentrated state of the pigment normally found in the lizard is due to the lack of the pituitary hormone in the blood.

Forbes (1937) found that prolonged injections of pituitary whole gland extract in the immature alligator, Alligator mississippiensis, cause the animal to become very dark.

Kleinholz (1940) and Kleinholz and Rahn (1940) used Anolis as a test animal for the chromatophorotropic hormone of the pituitary.

Although extensive work in this group has not been done, we see again that in all the investigated animals there is some trace of a pigmentary hormone in the pituitary gland. In this group, the hormone seems to have an important part in the normal color changes in the animal.



Intermedingehalt der Hypophyse in PE. bei Tier und Mensch.

Hypophyse	Intermedin in PE.
Elritze	7
Frosch	10
Huhn	75
Kaninchen	2—300
Hammel	2500
Rind	5—6000
Affe (Macacus rhesus)	1000
Mensch	4—7000

(Zondek and Krohn, 1932b)

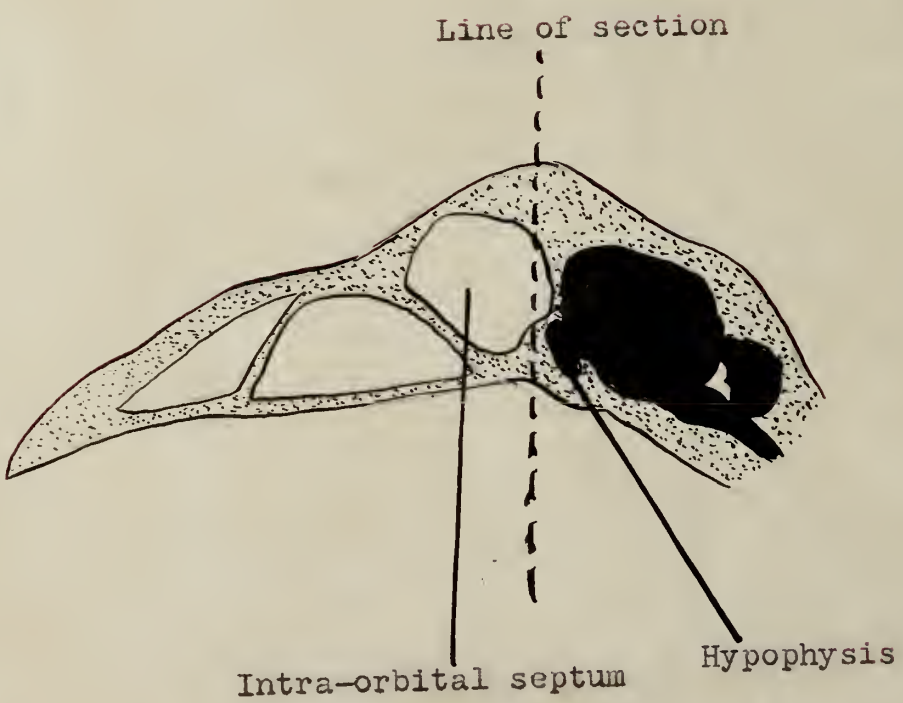
TABLE 1

Aves

This class has not been investigated to any great extent. There has been no work to determine the effect of the chromatophore activating substance on birds. All tests to investigate the presence of this substance in the warm blooded animals have been performed on cold blooded animals, as only the latter demonstrate color changes. Unless it is otherwise stated, light adapted or hypophysectomized frogs have been used.

Houssay and Ungar (1924b) reported the presence of a chromatophore activating principle in the hypophysis of the chicken, duck and pigeon. Zondek and Krohn (1932b) found that the pituitary of the chicken had about seven and one-half times as much of this substance as the frog, as can be seen in table 1. It must not be forgotten that the pituitary gland of the chicken is at least seven and one-half times as large as that of the frog.

Dietel (1933a) also reported the presence of an activator in the chicken, but whether or not this substance might also cause dilation of the blood vessels of the bird, was a question which he could not answer. However, this was probably the result of an incomplete separation of the melanophore and vasopressor principles. Kleinholz and Rahn (1940) found the pigmentary hormone in the chicken, also. Chen, Oldham and Geiling (1940) concluded that the hormone was present in the chick embryo as early as five days incubation. They found that injections of the pituitaries of 18 five day embryos caused hypo-



SAGITTAL SECTION OF CHICKEN SKULL WITH LOWER JAW REMOVED.

FIGURE 10

sectomized frogs to darken and stay dark for two hours.

The writer has found a very strong principle in the pituitary of the chicken. The chickens were procured from the various breeds which are found in an ordinary poultry market. The heads were removed from the chickens as soon as they were killed. It was found to be helpful to put the heads on dry ice and keep them there until the pituitary glands were removed. This prevented any possibility of autolysis. The pituitary was removed by cutting off the lower jaw and then sawing through the skull just a little in front of the sella turcica. The angle at which the cut was made can be seen on figure 10.

Although the number of species which have been tested in this group, are very small, here again, every animal which was tried showed the presence of a melanophore activating substance in the pituitary gland.

Mammalia

Oldham (1938) worked with the armadillo, Dasypus novemcinctus, which has no definite intermediate lobe. He found that the melanin dispersing hormone is present in the anterior lobe and in the stalk of the pituitary gland in this animal. Oldham, Last and Geiling (1940) confirmed this work.

Houssay and Ungar (1924b) first reported a chromatophore activating substance in the rat. Anderson and Haymaker (1935) grew the pituitary of the rat in vitro and found that these cells elaborated this substance. Geiling and Lewis (1935) used the same method and found the principle secreted by the cells of the hypophysis of the rat and the mouse.

Houssay and Ungar (1924b) found the substance in the guinea pig as did Collin and Drouet (1932).

In the rabbit, Hogben and Winton (1922b) Hogben (1924), Houssay and Ungar (1924b), Zondek and Krohn (1932b) and Jores (1933a) found the substance. The first four authors found this substance in the pituitary of the rabbit, but Jores found it in the lumbar fluid of this animal and also in the aqueous humor of a dark adapted rabbit.

Houssay and Ungar (1924b) reported this principle in the cat and the dog.

Hogben and Crew (1923) showed that the foetus of sheep of three months could produce enough of this material in its pituitary gland to darken a hypophysectomized axolotl. Houssay and Ungar (1924b) found the principle in the adult sheep. Zondek

and Krohn (1932b) also found it and estimated that the entire pituitary gland of the adult sheep contained about two hundred and fifty times the amount that the frog pituitary does (table 1). Popa and Fielding (1933) got a positive reaction which was very feeble and not always obtained. This disagreed with the results of the previous workers. Collin and Drouet (1934a) reaffirmed their own and others' results by producing blackening in hypophysectomized Rana tempoaria by implants of sheep pituitary.

Houssay and Ungar (1924b) detected this substance in the goat.

In the ox, the mammal which has been most used in these investigations because of its availability, the following workers found the pigmentary hormone: Hogben and Winton (1922b), Hogben and Crew (1923) on axolotl, Smith and Smith (1923a and 1923b) van Dyke (1926), Spaul (1928), Hogben and Gordon (1930), Collin and Drouet (1932). Zondek and Krohn (1932b) estimated that there was five to six hundred times the anount found in the frog pituitary. Veil (1937) was able to cause darkening in hypophysectomized cat fish. Kleinholz and Rahn (1940) also found the principle present in the ox pituitary .

Houssay and Ungar (1924b) found the substance in the horse and Blanchard, Prudhomme and Simonnet (1932) used the posterior pituitary extract of the stallion to cause diffusion of pigment in the melanophores of Acerina cernua and Gobio fluviatilis.

In the pig the hormone was discovered by Houssau and Ungar (1924b). Dietel found the hormone in large quantities in the pig liver (1931).

Zondek and Krohn (1932b) estimated that the pituitary of the ape (Macacus rhesus) contained about one hundred times the amount of the chromatophore activating substance which is found in the frog hypophysis. Ehrhardt (1932) also found it in the ape.

The chromatophore activating hormone has been noted in man by many authors. Hogben (1924) found it in a four month fetus. Dietel (1931) found the hormone in several organs of man, especially the liver. He also found traces in the human urine with a higher incidence in the female. Certain pathological conditions present large amounts of the hormone in the urine. Eclampsia is the outstanding disease in this group. Ehrhardt (1932) said that there was no more of the substance in the urine of pregnant women than of non-pregnant. This disagreed with some previous workers. Zondek and Krohn (1932b) stated that the amount of hormone in the human pituitary is four to seven hundred times the quantity in frogs. Jores (1933a) found the substance in the blood. He also found a substance in the urine which had a dispersing effect on the melanophores, but was not certain that this was the same as the hormone since it did not display all the chemical characteristics of the hormone. Drouet and Florentin (1935) could not get consistent results in pregnant women's urine, but did get positive results at times.

Valso (1934) found the secretion in the pituitary of the blue whale, Balaenoptera sibbaldi. Oldham, McCleery and Geiling (1938) found the chromatophorotropic hormone in the anterior lobe of the manatee, Trichechus inunguis. Fostvedt (1939) found the

same substance in the pituitary of the sperm whale. Oldham, Last and Geiling (1940) produced darkening in hypophysectomized frogs by injecting them with anterior lobe extracts from the pituitaries of the bottlenose dolphin, Tursiops truncatus, the finback whale, Balaenoptera physalis, and the white whale, Delphinapterus leucas.

Thus it has been shown that throughout the mammals, a chromatophore activating substance has been found. No animal in this class, which has been investigaed, has been found which lacks this substance. It should be noted again that in the warm blooded animals the only tests made for determining the presence of this hormone were done on the chromatophores of cold blooded animals. This makes more obvious the fact that we do not know the function of this substance in the former group of animals.

We have now seen that the pituitary gland of almost every animal (certainly every animal that has been tried in the vertebrates) secretes a principle which activates chromatophores. We shall now attempt to study this principle in order to discover where it is formed in the pituitary, what its characteristics are, and, if possible, what other effects it may have.

IN WHAT LOBE OF THE PITUITARY GLAND DOES THE CHROMATOPHORE ACTIVATING SUBSTANCE ORIGINATE ?

When Smith (1919a, 1919b) hypophysectomized his tadpoles, he did this by cutting away that part of the buccal ectoderm which was to be concerned in the formation of the pituitary. In other words, he removed everything except the anlage of the pars neuralis. From this we might say that since the tadpoles became lighter, the principle originates in the pars buccalis or in some structure that is derived from Rathke's pouch. However, these papers do not mention whether or not the pars neuralis developes normally without the other structures. It is possible that it does not, so we can not put too much stress on this line of reasoning.

Allen (1920) transplanted anterior pituitary lobes into his hypophysectomized tadpoles and found that they did not return to their normal color except for a slight temporary tendency at the beginning. He explained this by saying that there might have been a slight adhesion of particles from the intermediate lobe or a slight amount of diffusion from the latter lobe. The tadpoles became black after grafts of intermediate lobe or posterior and intermediate lobes were made. Swingle (1921) used only pars intermedia implants to darken hypophysectomized tadpoles. Atwell (1921) got this effect with posterior lobe extracts, but does not state the purity of his extracts. Hogben and Winton (1922b) used ox pituitary which is large and has easily separat-

ed lobes to investigate this question. They found that the anterior lobe gave no darkening, and neither did weak extracts of the pars nervosa, but strong extracts of this lobe and weak extracts of the pars intermedia gave marked darkening. They mentioned that this seems to disagree with Swingle (1921) who claimed that the pars intermedia had exclusive control of the pigment in the pituitary. Hogben and Winton said that their results may be explained by a very rapid diffusion from the intermediate lobe.

Smith and Smith (1923a, 1923b) disagreed with these authors and claimed that supposedly pure extracts of anterior lobe of ox pituitary caused a darkening of the skin in hypophysectomized tadpoles which might even extend to an unusual depth of pigmentation. Pars intermedia extracts also caused darkening, but posterior lobe extracts did this to a lesser degree. Houssay and Ungar (1924b) attempted to find where the chromatophore activating substance was present in the greatest concentration, by determining how much the extracts of ox pituitary could be diluted and still cause darkening. They found that one part of anterior lobe in five hundred of solvent, one part of intermediate lobe in four to five million and one part of posterior lobe in one hundred thousand were sufficient to cause darkening. This great difference in potency, especially between the pars intermedia and the pars anterior seems to point to diffusion from the pars intermedia. Hogben (1924) worked with frogs as his test animal as did Houssay and Ungar (1924b) and found that athough the pars intermedia was slightly more powerful that the

Abb. 1. X „weißer" Frosch.

(Bayer, 1930)

FIGURE 11

other lobes, all gave comparable results. However, the glands were obtained not less than three hours after killing.

Allen (1925) found that implants of adult frog pars intermedia in hypophysectomized tadpoles produced an animal which was much darker than the normal one. He concluded that this was because the gland of the adult was so much larger. He was not able to get darkening by implants of the anterior or posterior lobes. When dead pars intermedia was implanted, there was no result. From this he concluded that the phenomenon was caused by the living gland and not by absorption of stored secretions. This is a very significant paper in this series. Van Dyke (1926) found that the pars intermedia of the ox was especially rich in melanophore activating substance.

Allen (1929a) found that the pars intermedia gave , to a very slight extent, effects of the pars nervosa and that this lobe in turn exhibited some darkening in the hypophysectomized tadpole. He thought that this was due to a mutual diffusion between these two lobes. Pars intermedia transplants produced a pigmentation which became progressively darker until after three or four weeks it was intensely black, but pars nervosa only caused a slight darkening which disappeared in two or three days.

Bayer in a group of normal frogs in 1930, found one which was extremely light in color (figure 11). He examined the pituitary of the animal histologically and found that the intermediate lobe was atrophied and that only this lobe was affected. This was a fortunate find and must be considered very significant. This was one case where only the pars intermedia was altered and

the only apparent change in the animal is that it appears very light.

Allen (1930) took the precaution of taking his tissue for implants from regions of the lobes which were furthest from contact with other lobes. He, therefore, never got pigmentary effects with pars nervosa, and only slight transitory effect with pars anterior grafts. Pars intermedia caused a most intense darkening. He concluded that the pigmentary hormone is produced only by the pars intermedia. Since he used the very small pituitary of the adult frog, it is difficult to see how he could get absolutely uncontaminated slices.

Klatt (1931) worked with salamander larvae and found that posterior and intermediate lobe implantation gave the usual color effects, but anterior lobe gave none.

Zondek and Krohn (1932b, 1932d) compared the amount of this color affecting substance in different parts of the various lobes and came to the conclusion that, since the greatest concentration of the material was found in the intermediate lobe and since the concentration decreased in each lobe as samples were taken further and further away from the pars intermedia, the hormone is produced in the intermediate lobe. They named the hormone <u>intermedin</u>.

Noble and Richards (1932) found that implants of anterior lobe of adult Bufo fowlerii into Pseudotriton ruber gave a distinct reddening of the salamander. Pars nervosa and pars intermedia grafts produced marked darkening. He did not investigate this red color of the anterior lobe very carefully and we can

not be sure how it was produced.

Lundstrom and Bard (1932) found that removal of the neuro-intermediate lobe of the dogfish produced pallor in that animal, but extirpation of the anterior lobe did not have this effect. Posterior extracts of commercial preparations caused darkening, but anterior lobe extracts from the dogfish only a slight darkening.

Collin and Drouet (1934a) implanted pieces of anterior lobe, verified by histology, in Rana temporaria and produced an "intense" darkening which lasted only twenty-four hours.

Anderson and Haymaker's (1935) work showed that "pars intermedia cells of the posterior lobe retain their power to elaborate melanophore expanding principle" when grown in vitro. However, tissue cultures of pars anterior produced no discernible hormone. Geiling and Lewis (1935), who also worked on tissue cultures of rat pituitary, found that pure pars intermedia cultures produced large amounts of intermedin. Posterior lobe tissue mixed with intermediate lobe had both a blood pressure and a melanophore effect.

Atwell and Holley (1936) found partially hypophysectomized tadpoles which were silvery, but still metamorphosed. Histological studies of the animals showed that the pars intermedia was lacking in all cases. They concluded that the animals were albinous because the pars intermedia was not present.

Veil and May (1937) concluded from their work on the torpedo that the "neuro-intermediate lobe of the pituitary has an indisputable role in the coloration" of the fish.

Barry (1937) made a report similar to Bayer (1930). A light dogfish occuring in a group of normal dark dogfish was injected with an ox posterior pituitary extract. This made the fish as dark as the others when given in sufficient quantity. Anterior lobe extracts gave no results. Autopsy showed a peculiar pituitary. "The ventral nervous lobe with its connecting stalk and infundibulum seemed slightly smaller than usual in the fish; the vascular body (situated dorsally to the nervous lobe, between it and the brain) was smaller than usual, displaced laterally and seemed to be grafted onto the right angle of the optic chiasma. In the normal fish, the two lobes are in contact, but can easily be separated. In this one the gap is evident. Histologically, both lobes appeared normal, but in the vascular body, chromatophobe cells tended to prevail."

Although most of the work seems to point to the intermediate lobe as the source of this hormone, it is a fact that in some animals there is no distinct intermediate lobe. This has led some investigators to attempt to find the cell type which produces this substance. The conclusions of these workers are not definite enough to solve this problem, but some of the more recent experimenters will be included here. Oldham, McCleery and Geiling (1938) found the anterior lobe of the manatee to be rich in the melanophore activating substance. Oldham (1938) found the same state in the armadillo which also has no intermediate lobe. Kleinholz and Rahn (1940) determined that the cephalic third of the anterior lobe of the chicken pituitary contained twenty times as much intermedin as the caudal third. They ex-

amined the former portion histologically, but found at least five cell types in this region and could not conclude which one was most important in secreting intermedin. They decided to attempt to correlate the developement in ontogeny of the pituitary gland with the first appearance of intermedin. They used the frog for this. Kleinholz (1940) reported the results and stated that intermedin appeared before there was any apparent differentiation of cells in the pituitary gland. It may be that his methods were not effective enough to demonstrate any differentiation or it may be that there is physiological differentiation before histological changes can be seen.

Chen, Oldham and Geiling (1940) found that intermedin first appeared in the chick of five days incubation and at this time the anterior lobe no longer appeared as a simple evagination of the buccal epithelium. They say, "In the five day embryo, the proximal end of the buccal evagination is constricted and attenuated, while the walls of the distal portion have become greatly thickened, with bud like outgrowths extending into the surround-mesenchyme. The neural lobe appears for the first time as a small evagination of the infundibulum which comes in close contact with the tip of the anterior lobe." Oldham, Last and Geiling (1940) found that the cetacean and armadillo hypophysis contained the melanophore dispersing hormone in greatest concentration in the antero-ventral region of the anterior lobe.

In all of the work which has been done, the possibility of diffusion and its effect has come up. Some of the previously mentioned workers spent some time on this question. They are

Allen (1929a, 1930), Zondek and Krohn (1932b), Collin and Drouet (1932), and Anderson and Haymaker (1935). However, Spaul (1928) made a careful study of this particular problem with significant results. He used ox pituitary and divided it into anterior and posterior lobes. He found that, " the pigment factor diffuses from the posterior lobe into the anterior lobe, the rate decreasing as the difference between the amounts present in each lobe decrease until the effects of autolysis become apparent when its destruction proceeds in both lobes. The rate of diffusion increases, but the period during which it is able to mask autolysis decreases with the temperature. The presence of other principles and of autolytic products does not affect the response of the pigmentation principle. Diffusion and autolysis are responsible in a large measure for the variations in the metamorphic and pigment results obtained by the administration of anterior and posterior lobe products."

This last sentence is important because it states what workers have long suspected. From the results which have been presented in this paper, it must be concluded that this is at least a very probable explanation for discrepancies between different workers.

It has been pointed out before in this paper that the pituitary gland is not the only place where intermedin has been found. Dietel (1931), as has been noted, found it in many organs of man and animals, in still-born children, and especially in human and pig livers. To a lesser degree, he found it in human urine, with slightly more in the female than the male. Ehrhardt (1932)

said that the hormone was not found in any great amounts in tissues of the human body, except in the placenta in eclampsia. He attempted to discover what would happen to the hormone if it were given orally to patients and could not get blood or urine from these persons to darken frogs. This might have been due to the insensitivity of the test. Zondek and Krohn (1932b) found slight traces of intermedin in the tuber cinereum and hypothalamus of man. They also found a trace in the proventricular central gray matter of the thalamus, but in no other parts of the brain. They could find no trace in the liquor of the fourth ventricle or in the lumbar fluid of the human, although at times they could detect minute quantities in the fluid of the third ventricle. Jores (1933a) found intermedin in the human blood, but stated that the substance which he found in the urine did not possess the chemical characteristics of the hormone although it did have a pigment dispersing effect in the melanophores.

Since this hormone is the secretion of an endocrine gland, it is natural that it be found in the body tissues and organs to some extent. It has been found nowhere in as large quantities as in the intermediate lobe of the pituitary. The work of these investigators on hypophysectomy and pituitary therapy, culminated by the tissue culture experiments, points to the conclusion that the melanophore and chromatophore activating substance of the pituitary gland is synthesized by intermediate lobe cells. Some observers have attributed other functions to this hormone, but these will be discussed with the chemistry of

the hormone. This would be, however, a good point at which to mention briefly the opinion of several men on the mode of action of the hormone.

Spaeth (1913 and later) in his work on isolated melanophores was instrumental in showing that the pituitary substance could have effect on a denerated cell. This did not demonstrate anything about the normal mode of action. Smith (1919c) made skin transplants between normal and albinous tadpoles. The skin which differed at first in color from the rest of the integument of its new host soon had the identical tint (Figure 8). This showed that the substance in the frog acted directly on the cells and it probably did this in the normal animal. Smith (1920) said that the melanophore and xantholeucophore changes in the albino were not due to alterations in their nervous mechanism, but to the modified tissue fluids which bathe them. Hogben and Winton (1922b) proved this in a different way when they used various drugs to paralyze different types of nerve endings in the frog and still got a darkening with intermedin. Isolated skin in their experiments also reacted. They, too, concluded that the action of the hormone was probably directly on the melanophores.

It is interesting to note that Houssay and Ungar (1924b) got a response when the tadpole was immersed in a pituitary solution only when the concentrations were extremely strong. It is evident that the selective permeability of the skin was a factor here. They concluded that the action was direct. Giersberg (1931) concluded that the seat of action of Infundin was directly

on the cytoplasm of the chromatophores. Their work was done on Phoxinus. Peczenik (1933) thought that intermedin worked in Phoxinus by means of a "central attack on a spinal vegetative mechanism", as well as by direct peripheral action.

Veil and May (1937) concluded their work on the Torpedo by saying that they thought the dispersion of the pigment in the melanophores in this fish was due to the irrigation of these cells by blood containing the pigmentary hormone. All the evidence seems to show that the hormone acts directly on the chromatophores. There is not much doubt that in the teleosts and perhaps in the elasmobrachs there is an additional nervous control, but the hormone acts without the mediation of nerves.

THE CHEMISTRY OF INTERMEDIN

Identity of intermedin.

A good deal of the difficulty in the determination of intermedin as a separate entity came from a confusion which led investigators to believe that it was identical with the oxytocic and pressor substances of the posterior lobe. This was natural since it was not until comparatively recently that workers got the pars intermedia pure enough to have no diffusion from the posterior lobe. There are, however, definite chemical and biological facts which demonstrate the individuality of the melanophore hormone.

Hogben and Winton (1922b) found that intermedin retains its potency even after boiling for half an hour in 0.5 percent HCl. This separates it from the pressor substance, since the latter is rapidly destroyed by acid hydrolysis. Hogben and Gordon (1930) found that subjecting pituitary extracts to prolonged action of NaOH destroyed the pressor activity, but increased the melanophore activity of the extracts. They held that the melanophore, pressor and oxytocic activities of the extracts of the pituitary gland are referable to distinct entities, but did not exclude the possibility that they were all different radicals of the same molecule. They thought perhaps the method of separation which is mentioned above merely split a complex molecule. Zondek and Krohn (1932c) got an extract of the pituitary gland

which displayed only chromatophore activity. They stated that this showed that intermedin was a separate hormone.

By reasoning from the biological characteristics of the pituitary hormones, several investigators were able to show that intermedin was not the same as other pituitary hormones. Van Dyke (1926) pointed out that since the pars neuralis is far richer in uterus-affecting, blood pressure-raising and anti-diuretic substances than the pars intermedia and since the latter lobe is extremely rich in the chromatophorotropic component, it is unlikely that intermedin could be the same as any of the other three mentioned. Lundstrom and Bard (1932) were unable to produce the pigmentary effect with the oxytocic principle of the preparation, Pitocin. Thus it could not be the same as the chromatophore activating principle.

Zondek and Krohn (1932b, 1932d) found the principles isolated in the normal individual. They reported the following: "Oxytocin, Vasopressin und Intermedin verlassen die Hypophyse auf dem Wege über den Hypophysenstiel. Während Oxytocin und Vasopressin in den Liquor des 3. Ventrikels übergehen und in der Zisternenflüssigkeit wie im Lumbalpunctat nachweisbar sind, findet man das Intermedin nur am Ort seiner Wirkung, d.h. nur in der Wand des 3. Ventrikels." Since intermedin is not found where the other two hormones are, it can not be the same as either of them. Zondek and Krohn (1932c) further substantiated this work when they found that in the colloid of the pituitary, intermedin was present, but not oxytocin. They also mentioned the fact that the different principles were found in varying proportions in the lobes of

the pituitary. Geiling and Lewis (1935), in their tissue culture experiments, found that the chromatophore activating substance from the secreting cells of the pars intermedia possessed no blood pressure raising property.

Chemical characteristics of intermedin.

Hogben and Winton (1922b), in addition to finding that intermedin can withstand acid hydrolysis, also found that it was destroyed by trypsin, but not affected by pepsin digestion.

Zondek and Krohn (1932c, 1932d) disagreed with this. They found that intermedin can stand cooking, and that low temperature of -220° C has little effect. It is sensitive to ultraviolet rays and 95 percent of it is destroyed after thirty minutes exposure to a quartz lamp 15 centimeters away. Pure intermedin is 50 percent soluble in 100 percent ethyl alcohol, but not at all in 30, 50 and 70 percent. It is fairly soluble in methyl, propyl and butyl alcohol, but insoluble in benzol, chloroform, ether, acetone and acetic ether. The solubility in alcohol depends on the purity of the intermedin. It is entirely adsorbed by charcoal and kaolin, but only 90 percent adsorbed by infusorial earth, 80 percent by a Berkefield filter, and 50 percent by $Fe(OH)_3$ and $Al(OH)_3$. Only about ten percent of the raw pituitary extract can be recovered after precipitation in heavy metal salts. Probably most of the intermedin is destroyed. It is fairly stable in acids and bases of up to four percent concentration. A water solution of intermedin dialyses about 15 percent.

This is not very large recovery, but since the oxytocic and vasopressor principles can only be recovered up to 3.3 percent, it is of value in separating them. In the blood of warm blooded animals, intermedin is decomposed very rapidly.

Dietel (1933a) concluded, since the melanophore hormones of poikilothermic and homoiothermic animals are identical in their effect on the frog, they were the same chemically. This could hardly be called a valid conclusion. Dietel (1933b) made the following observations which agree closely with those of Zondek and Krohn. The melanophore hormone is poorly soluble in butyl alcohol and more easily soluble in ethyl alcohol than the other components, and precipitated by acetone. It has great insensitivity to alkali, unlike the uterine, blood pressure and intestinal stimulating principles which are very sensitive. It diffuses poorly through collodium filters. Dietel adds that he is not sure whether the separation of intermedin from oxytocin and vasopressin is due to the method of extraction or whether the separation is a natural one. Previous discussion points to the probability that the separation is a natural one.

Peczenik (1933) was able to cause an increase in the intermedin effect by means of the addition of Na_2PO_4 or grape sugar. He does not go into enough detail to make clear just how this reinforcement is accomplished. Jores (1933a) stated that the substance in the human urine which gives the intermedin effect does not have the chemical characteristics of the hormone.

Koller and Rodewald (1933) found that the hormone from the pituitary of dark adapted frogs did not darken pale frogs.

Jores (1934) also found that this was true, but while the former authors exposed their frogs to light for a few minutes to reactivate the hormone, the latter writer found that he could accomplish this by alkali extraction of the extract from a dark adapted frog. Jores saw that there were several possible explanations of this. He mentioned three. 1. The increase in potency of the extracted intermedin over the raw is due to the destruction of a counteracting chemical. 2. A chemical change takes place in which the hormone is transformed to an effective state. 3. An inactive pre-hormone is activated. This last statement brings up the possibility that there may be a substance in the pituitary gland which extraction methods make into a melanophore activating principle, but which is not transformed into intermedin in the normal animal. This will become more significant in the discussion of extraction methods.

Kleinholz and Rahn (1940) stated that the amounts of intermedin from the pituitary glands of light-adapted frogs and of frogs kept in darkness do not differ significantly.

Parker (1935a) mentions something which has been slighted because it is so obvious, but which, nevertheless, should be mentioned. It is that, since the hormone is contained in the blood, it must be water soluble.

Methods of extraction.

Obviously the methods of extraction depend on the known chemical properties of intermedin. Since actual use of these

methods requires a fairly intricate technique, only the salient points of the various methods will be mentioned. In 1930, Hogben and Winton extracted the melanophore principle by subjecting pituitary extracts to prolonged contact with NaOH. This caused the destruction of the pressor activity, but also caused an appreciable increase in melanophore activity. They explained this increase by saying that the removal of the pressor principle allowed the blood vessels to dilate and therefore more of the intermedin could get to the chromatophores.

Zondek and Krohn (1932c, 1932d) observed that intermedin could be extracted almost quantitatively by 0.25 percent of acetic acid. They stated that certain precepts should be observed in the extraction of intermedin. It should be produced in the highest concentration or an albumin free solution with the least amount of water, and it must be free from oxytocin and vasopressin. They used the anterior lobe for the raw material since it contained much more intermedin than oxytocin or vasopressin. They extracted with sulphosalicylic acid or trichloracetic acid and obtained a solution rich in intermedin, but with traces of oxytocin and vasopressin. They removed these traces by extracting in alcohol. They prepared their raw gland by drying it in acetone until the acetone was colorless.

Matthews (1933) merely ground the whole pituitary in 0.5 cc of 0.1N NaCl. Dietel (1934) devised a method of extraction with $Ba(OH)_2$ and purification which gave a purer extract than by Zondek and Krohn's method. By his method, one frog unit was equivalent to 0.0003 gamma and with their method, one frog unit

was equal to 0.01 gamma. Stehle (1936) reported a method involving the vacuum desiccation of an alcohol extract which is further purified by alcohol extraction. This does not produce as good a yield as either of the two above methods. Kleinholz (1940) used an extract prepared by boiling the dried pituitary in 0.1N NaOH and then neutralizing it with 0.1N HCl.

Methods of assay of intermedin.

The first attempts to assay the melanophore activity of pituitary hormones was made by Spaeth (1916b, 1918) on isolated scales of Fundulus. Although this method is very convenient, it is not very reliable and has given way to the methods of other investigators. Fenn (1925) perfused pithed frogs through the bulbus arteriosus with Ringer's solution until the skin was completely paled. He then tied off one fore and one hind leg as controls and continued to perfuse his frog with the test liquid in Ringer's solution. The slightest amount which could be detected was called a frog unit. In the early work with this hormone, several workers including Loewe and Ilison (1925) and Rowe (1928) proposed using this method to assay the active principles of the pars neuralis. It has been shown why this would be impractical. Snyder (1928) used a perfusion method similar to that of Fenn.

Zondek and Krohn (1932a, 1932d) felt that the melanophore reaction was not specific enough to be a good test for intermedin. (Other drugs beside intermedin may cause dispersion of the pigment in the melanophores.) They proposed the use of the

erythrophores of male Phoxinus of certain sizes as a test and they found no other drug or hormone among those they tried which caused the pigment in the erythrophores to disperse. The minimal effect which was produced by a dose of one Phoxinus unit, P. E. (Phoxinus Einheit), is a reddening around the base of the pectoral, ventral and anal fins.

Teague, Noojin and Geiling (1939) did not agree with Zondek and Krohn and other workers. They said, "The effect of a number of substances upon the melanophores of normal and hypophysectomized frogs was investigated. In normal frogs, darkening and melanophore expansion was produced with posterior pituitary extracts, brucine, veratrine, metrazol, pilocarpine HCl, yohimbine HCl, chloretone, chloralose, methane, avertin and some of the barbiturates. A large number of other substances, many of which have been reported to darken frogs, failed to darken either normal or hypophysectomized frogs. No substance studied except posterior pituitary extract was found to darken or produce melanophore expansion in hypophysectomized frogs. Hence the hypophysectomized frog, Rana pipiens, is a specific test animal for the melanophore hormone. Conflicting results with other authors may be due to species difference."

Kleinholz (1940) and Kleinholz and Rahn (1940) used, as their method of assay, hypophysectomized Anolis carolinensis into which was injected the test material. They made a scale of five stages of color range of the dermal melanophores. The amount of intermedin which would bring the animal to tint one was designated as one Anolis unit. That which would cause tint

number two was two Anolis units and so forth.

Since all the methods for assay are biological, they can not be considered truly quantitative. An accurate and infallible test for intermedin can only be devised after its chemical nature is known.

DISCUSSION

The question of the effects of the hormone, other than that of dispersal of pigment of chromatophores, is properly taken up in the discussion since, for the most part, there is little conclusive evidence. As has been mentioned previously, Smith (1919a) said that hypophysectomized tadpoles are made lighter, not only by the concentration of pigment in the melanophores, but also by a reduction of the number of these cells and by a pigment poor condition in the cells. There was also a marked reduction in the amount of free melanin granules in the epithelium. Atwell (1919) said that pigment cells may become invisible by their migration from the epidermis to deeper parts, or by the loss of pigment. These results were confirmed by Smith (1920) and Atwell (1921). Allen claimed that pituitary grafts in the hypophysectomized tadpoles, in addition to the other effects, caused a deposition of pigment granules. This confirmed the above work.

Zondek and Krohn (1932c, 1932d) said that red pigment could be extracted from the skin of Phoxinus, especially by chloroform, more easily when intermedin had been injected into the animal than in the control. The pigment not only went into solution quicker, but more could be extracted. This might mean that intermedin not only causes dispersion, but also may cause the formation of the red pigment. The conclusion of all these papers is about the same. Intermedin may actually cause a

formation of new pigment in addition to affecting the pigment already present. It may cause the migration of pigment from deeper layers to the surface.

Whether or not the product obtained by extraction of the pituitary gland is actually something in the gland or whether it is produced from something else by the method of extraction, is not determined. The synthesis may be the same as would occur in nature, but is all the material extracted available to the animal under normal conditions? This question has not been answered and probably can not be until the chemical structure of intermedin is known. Until this is found, we can not say whether or not the substance found in the urine by several investigators in actually intermedin.

It is quite certain that intermedin has some effect on the deep melanophores of amphibia. It also affects the pigment cells of the retina of the frog as mentioned by Dubois-Poulsen (1925), Jores and Caesar (1935) and Matuo (1935). Jores (1933b) found that intermedin injections shorten the time for dark adaption in the human eye. He also found that the hormone content in different species has a definite relation to the vision of the animal in the dark. These papers point quite definitely to a possible function of intermedin in the warm blooded and the cold blooded animals. This is a field on which more work should be done in order to solve the enigma of an apparently functionless hormone in the warm blooded animals. It seems to author that this is a very likely function of intermedin in homoiothermic animals.

Dietel(1933a) and others have attributed other functions, such as vascular effects, to intermedin. It is most probable that in these cases the substance was not pure. Careful studies show that the hormone probably does not have the same function as any other pituitary hormone. Allen (1928) showed that intermedin has no effect on growth Zondek and Krohn (1932c, 1932d) found that intermedin has no effect on the following: the thyroid gland, growth, sex, metabolism, uterine musculature, blood pressure, intestinal and bladder musculature, antidiuresis, blood sugar, and liver function. This work was done after careful extraction of intermedin from the other pituitary principles. Dietel (1933b) refuted what he had said in 1933a and stated that intermedin shows no reactions of the other hormones of the pituitary gland. This does not imply that all the functions of the pituitary hormones or intermedin are known.

There are some peculiarities in the work with intermedin which are worth mentioning again. Collins and Adolf (1926), working on the vermillion spotted newt, found that Pituitrin fed to both dark and light individuals caused the dark ones to lighten. The same thing occurred with injections. This is very strange especially since the work on amphibia was so consistent. Witschi (1931) found that incomplete hypophysectomy on Triturus torosus caused the animal to get darker than normal. He explained this by saying that it was due to a hypertrophy of the hypophysis. These two latter experiments should be reinvestigated.

In one experiment, the writer produced pallor in frogs after injecting them with a pituitary extract from dark adapted frogs.

In two subsequent experiments, however, the test animals got darker after injection. Since the extracts were always made from the pituitaries of at least four or five dark adapted frogs, these results are difficult to explain. Probably some factor which is of importance has been overlooked, although the length of time of dark adaption and the temperature of the animals from which the extracts were obtained were ruled out. The test animals were always in similar conditions in each experiment.

There is also the unsolved problem of whether or not there need be an antagonistic mechanism to the intermedin reaction. The fact that the melanophores and the xantholeucophores react in opposite ways to intermedin might point to the possibility that antagonists are unnecessary. On the other hand, if intermedin makes the pigment disperse, why should the pigment concentrate when no hormone is present? One of the body hormones, namely adrenalin, has the opposite effect to intermedin, but isolated skin and scales which have no source of adrenalin contain cells whose pigment responds when intermedin is removed. This seems to conflict with the viewpoint of some investigators that there must be an antagonist. Fish chromatophores are definitely innervated, but what of the other classes and of the free pigment granules?

Kropp and Perkins (1933a, 1933b), and Perkins and Kropp (1932) have shown that crustacean eye stalks produce a substance which has an effect on poikilothermic vertebrates which seems identical to that of intermedin. It would be interesting to investigate their chemical similarities. Eye stalks of Fundulus

do not contain this substance. It may be that the pituitary gland has taken over the function of the eye stalks in the vertebrates.

Spaeth (1916a) and Wyman (1924) presented the hypothesis that the melanophores are modified smooth muscle cells. This is very interesting when we consider that two similar hormones in the pituitary gland, oxytocin and vasopressin are both modified substances for governing somewhat altered smooth muscle cells.

Hewer (1923) showed that dryness and light cause concentration of pigment in the melanophores of amphibia, and moisture and darkness cause the opposite effect. Since the environment in which the frog lives during its mating season is dark and moist, this alone might account for the dark mating costume of the frog. At anyrate, how are heat and light translated into hormone flow?

Schürmeyer (1926) found that damage to the floor of the third ventricle causes light adapted frogs to darken. Perhaps nerve fibers from this region cause the pituitary to secrete. Wunder (1931) and others have worked with the "wedding-dress" of the minnows. In these animals there seems to be no environmental changes in the laboratory which initiate these changes, but the alterations occur nevertheless. The inevitable question of why these changes should occur at all arises, too. Parker (1930) went into a long discussion of the problem, but since all treatments of this are filled with speculation and rationalization, there is little point in repeating them here.

The very important aspect of the application of this knowledge to human problems is filled with contradictions and speculations on the part of the experimenters. Zondek and Krohn (1932c, 1932d) close their work with the following comments. Marked pituitary changes occur during pregnancy when there are pigment changes as well. In some pituitary changes there is a depigmentation of the genito-anal region. The adrenal gland was credited with these chages previously. Perhaps the pituitary gland works directly on these pigments as well as through the adrenotropic hormone.

Ehrhardt (1932) reported that for unknown reasons, the pituitary of a man who had died of cirrhosis of the liver contain- no intermedin. Dietel (1933b) said there was more intermedin in the serum of gravid females than normal. Drouet and Florentin (1933) got varied results on the same woman on succeeding days, although she received no treatment of any kind. On one thing all the writers agree. The urine and the placenta in cases of eclampsia contain enormous amounts of intermedin.

Dietel (1933b) injected extremely large amounts of pituitary extracts into humans and produced pigmentation in them. This was caused by a formation of pigment rather than alteration of pigment cells.

The full significance of intermedin is certainly not known. This is especially true in the warm blooded vertebrates. As Professor Lutz has pointed out, it is unlikely that the pituitary gland should continue to secrete a hormone, in relatively large amounts, after the substance is no longer functional. It is

most probable that intermedin is important in the dark adaption of the human eye and possibly also in minor pigmentary changes.

The author would like to take this opportunity to thank Professor Lutz for the many suggestions and helpful criticisms which he has offered in the writing of this paper and in the planning of research in this field.

CONCLUSIONS

1- It has been shown that in the intermediate lobe of the pituitary gland of a great many vertebrates, a substance is produced which has a definite effect on the chromatophores of poikilothermic vertebrates.

2- This substance is called intermedin, and it is not the same as any other pituitary hormone either in its chemical characteristics or its physiological effects. The chemical nature of this hormone is not known.

3- Intermedin may have other important effects, such as a role in the migration of the retinal pigment.

4- In fishes, the nervous system is of prime importance in controlling the chromatophores, while in amphibia and reptiles, intermedin predominates in control.

5- Effects of intermedin on homoiothermic vertebrates are not definitely known at the present time.

ABSTRACT

This paper is concerned with the color changes of the cold blooded vertebrates in which the pigment cells of the integument are concerned. It was first thought that these pigment cells or chromatophores actually contracted and expanded, amoeba-like, during alterations of tint. Careful studies showed that in the melanophores or black pigment cells, this was not the case. Here the pigment granules migrated within the clear protoplasm of a cell with fixed boundaries. This led to a change in the nomenclature from "expansion and contraction" of the chromatophores to "dispersion and concentration of the pigment" of these cells. The variations in the position of the pigment in these tiny cells account for the color changes which can be noted with the naked eye.

In attempting to find what caused the pigment of these cells to migrate, experimenters worked extensively on fish. In the elasmobranchs, it was noted that removal of the pituitary gland caused a fish to pale because of a concentration of the pigment of the melanophores in the integument. The nerves seemed to play only a small part here although some investigators placed more emphasis on nervous control than others. In teleosts, the nerves were found to have a very important part in the control of chromatophores. However, the hormone of the pituitary gland had effects despite the innervation.

In amphibia, the hormone played a bigger part because there was no nervous mechanism of any importance. In the frog, the importance of the melanophores was slightly eclipsed by the xantholeucophores. These golden white cells covered the melanophores beneath them when their pigment was dispersed. It was these cells which gave the lustre to hypophysecomized tadpoles. In the latter tadpoles, called albinos, the pigment of the melanophores was concentrated and that of the xantholeucophores was dispersed. This effect was reversed by pituitary injections and by implantations. Essentially the same was true in the reptiles, except that, in this class, the melanophores were most important.

Several investigators have found the pituitary substance, which affects the chromatophores of the cold blooded animals, in the hypophysis of the bird and mammals where-ever the pituitary was investigated.

It was discovered that this hormone was secreted by the intermediate lobe of the pituitary gland and for this reason, the hormone was named intermedin. Intermedin was found to be a substance with no biological properties like those of any of the other pituitary hormones. It was found to have unique chemical characteristics also. It had a chromatophore activating effect in cold blooded vertebrates and a possible retinal pigment effect.

Methods of extraction of the hormone were based on its chemical properties although its exact chemical nature is not known. Investigators used bio-assay to determine the presence of this hormone.

Although the hormone is present in warm blooded animals, it is not clear what its function is. It may have some connection with the retinal pigment or it may have some effect on pigmentation in these animals.

The only safe conclusion which can be drawn is that there is a hormone of the intermediate lobe of the pituitary gland of the vertebrates which has an effect on the chromatophores of the cold blooded vertebrates and that this hormone has certain chemical and chemical characteristics which are unique.

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